Human Langerhans Cells Express a Novel Form of the Leukocyte Common Antigen (CD45)

Gary S. Wood, Paul Szwejbka, and Anita Schwandt

Departments of Dermatology and Pathology, and the Skin Diseases Research Center, Case Western Reserve University, and the Veterans Affairs Medical Center, Cleveland, Ohio, U.S.A.

CD45 is a family of transmembrane glycoproteins that function as protein tyrosine phosphatases. All isoforms exhibit common CD45 epitopes, whereas the restricted CD45 epitopes (RA, RB, and RO) are each limited to one or more isoforms. In prior studies, we showed that human Langerhans cells in normal epidermis express a novel CD45 phenotype. They express common CD45 epitopes but are characteristically $RA^-RB^-RO^-$. This suggests that Langerhans cells can express a novel form of CD45. In order to clarify this issue further, mRNA extracted from enriched Langerhans cell preparations was reverse transcribed into cDNA. The 5′ portion of CD45 cDNA was then amplified using polymerase chain reaction primers complementary to exon 2 and exons 9–10, which flank the CD45 variable exon region (exons 4–6). Cloning and sequencing of the dominant 441 bp polymerase chain reaction product revealed the following exon configuration for the 5′ translated region of Langerhans cells CD45: exon 3/7/8/9/10. This is the same exon configuration associated with the 180 kd CD45 isoform expressed by memory T cells and monocytes/macrophages; however, these cell types are RO^+ whereas normal Langerhans cells are RO^-. The RO epitope is known to be an oligosaccharide with a terminal sialic acid moiety. Therefore, we determined the expression of a related epitope, OPD4, by Langerhans cells. This is another terminal sialic acid moiety expressed by the 180 kd CD45 isoform of memory T cells but not by monocytes/macrophages. Langerhans cells were $OPD4^-$. Our data suggest that memory T cells, monocytes/macrophages, and Langerhans cells all express a common CD45 transcript lacking exons 4–6; however, this transcript appears to undergo lineage-specific, post-translational glycosylation to create three distinct CD45 glycoproteins: RO^+OPD4^+, RO^+OPD4^-, and RO^-OPD4^-, which are expressed typically by memory T cells, monocytes/macrophages, and Langerhans cells, respectively. Because these epitopes are located extracellularly, they are postulated to allow differential responses to extracellular stimuli by creating differential ligand specificity. ***Key words: protein tyrosine phosphatase/RT/PCR.***

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The human CD45 antigen consists of a family of heavily glycosylated transmembrane proteins, one or more of which are expressed by all bone marrow-derived cells of leukocytic lineage, including Langerhans cells (Wood *et al*, 1985a; Cobbold *et al*, 1987; McMichael, 1987a, b). CD45 molecules function as protein tyrosine phosphatases (PTPases) involved in the positive regulation of T cell and B cell antigen receptor signaling via activation of Src family kinases (Cahir McFarland *et al*, 1997; Ulyanova *et al*, 1997; Wallace *et al*, 1997). Through this and other mechanisms, CD45 has been implicated in a variety of leukocyte functions including thymocyte selection and maturation, cytotoxicity, apoptosis, expression of interleukin-2 receptor and L-selectin, suppressor inducer function by $CD4^+$ T cells, immunoglobulin production, T cell trafficking, and neutrophil chemotaxis (Shen *et al*, 1985; Saga *et al*, 1986; Cobbold, 1987; McMichael, 1987a, b; Koretsky *et al*, 1991; Stover *et al*, 1991; Alexander *et al*, 1992; Streuli *et al*, 1992; Conroy *et al*, 1996; Kozieradzki *et al*, 1997; Latinis, 1997; Wang *et al*, 1997; Wroblewski, 1997). Furthermore, it has been proposed that the contact inhibition of cell division and motility may be mediated by transmembrane molecules with CD45-type intracellular domains (Streuli *et al*, 1989). In this context, CD45 has been associated with the cytoskeletal component fodrin (Suchard, 1987). The B cell antigen, CD22, has been identified as a ligand for CD45RO, providing additional evidence that CD45 molecules are involved in cell–cell interactions (Stamenkovic *et al*, 1991).

There are multiple isoforms of human CD45, all encoded by a single gene that is situated on the long arm of chromosome 1 in the q31–32 region (Ralph *et al*, 1987; Streuli *et al*, 1987, 1988; Hall *et al*, 1988; Streuli and Saito, 1989; Ratech *et al*, 1997). There are 33 exons that encode the entire cDNA sequence of the largest CD45 isoform, including 5′ and 3′ untranslated regions. The human CD45 isoforms can be segregated into four glycoprotein bands (180 kd, 190 kd, 200–210 kd, and 220 kd) by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The exon organization of these isoforms determines their molecular weight and is associated with specific patterns of CD45 epitope expression (**Table I**). This occurs by alternative splicing of CD45 mRNA involving exons 4, 5, and 6 (the three known variable exons in humans).

In 1984, we reported that human and rodent Langerhans cells express CD45 based on their reactivity with anti-CD45 monoclonal antibodies at both the light microscopic and the ultrastructural levels (Wood *et al*, 1984, 1985b). Subsequently, we used two-color epitope mapping to show that Langerhans cells in normal human epidermis express a unique CD45 phenotype: $CD45^+RA^-RB^-RO^-$ (Wood *et al*,

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Reprint requests to: Dr. Gary S. Wood, Chief, Dermatology Service, VA Medical Center, 10701 East Blvd., Cleveland, OH 44106.

Table I. Molecular and antigenic characteristics of six known human CD45 isoforms[a]

Exons in mRNA	Protein molecular weight	CD45 common	CD45RA	CD45RB	CD45RC	CD45RO
1–2–3–4–5–6–7–etc.	220 kd	+	+	+	+	–
1–2–3–4–5–7–etc.	205 kd	+	+	+	–	–
1–2–3–5–6–7–etc.	200 kd	+	–	+	+	–
1–2–3–4–7–etc.	190 kd	+	+	–	–	–
1–2–3–5–7–etc.	190 kd	+	–	+	–	–
1–2–3–7–etc.	180 kd	+	–	–	–	+

[a]Based on the data of Streuli *et al* (1987, 1988) and Ratech *et al* (1997). Potential isoforms encoded by transcripts containing only exons 4 and 6, or exon 6 alone, in their variable regions have not been identified in humans. Multiple antibodies specific for CD45 common, RA, RB, and RO epitopes have been described. Antibody YTH80.103 has been reported to have putative specificity for CD45RC (exon 6-encoded epitope) (Schwinzer *et al*, 1995).

1991). This suggested that they express a novel CD45 isoform because all known isoforms contain at least one restricted epitope (RA, RB, or RO) (**Table I**). Two major possibilities existed. First, Langerhans cell CD45 might have a novel exon configuration and primary protein structure. This novel structure would then result in a novel pattern of CD45R epitope expression because most CD45R epitopes are known to be, or to be closely associated with, O-linked oligosaccharides whose presence correlates with the presence of specific CD45 exons: CD45RA (exon 4), CD45RB (exon 5), CD45RO (exon 3/7 junction) (Barclay *et al*, 1987; Streuli *et al*, 1988; Pulido, 1989; Poppema *et al*, 1991; Wood, 1991; Knowles *et al*, 1992; Saunders *et al*, 1995). An alternative possibility was that Langerhans cells might express one or more of the known CD45 transcripts, but that this message might undergo a novel form of post-translational processing so that it would exhibit a different pattern of O-linked oligosaccharides and therefore a novel pattern of CD45 epitopes. For example, this type of differential, cell-specific glycosylation is known to occur with the CD43 molecule (Peacocke, 1992).

MATERIALS AND METHODS

Immunoperoxidase analysis Immunostaining of cryostat sections of skin, epidermal strips, and cytocentrifuge preparations of enriched Langerhans cells were performed as described previously (Wood, 1982) using a three stage method consisting of (i) murine monoclonal antibody, (ii) biotin conjugated $F(ab')_2$ fragments of goat anti-mouse IgG, and (iii) avidin-horseradish peroxidase. The chromagen was 3,3-diaminobezidine. Controls included irrelevant primary antibodies of similar isotype and staining with various stages deleted.

Monoclonal antibodies Several monoclonal antibodies were used for immunostaining of cryostat sections of intact skin and epidermal strips, cytocentrifuge preps, and western blots. These included NA1/34 (CD1a) (Dako, Carpinteria, CA); Leu4 (CD3), LeuM3 (CD14), Leu14 (CD22) (all from Becton Dickinson, San Jose, CA); CD45 common antibodies 2D1 (Dako), T29/33 (Boehringer Mannheim, Indianapolis, IN), and BMAC3 (Rosemarie Dalchau, Sussex, U.K.); CD45RA antibody Leu18 (Becton Dickinson); CD45RB antibody PD7/26 (David Mason, Oxford, U.K.); CD45RO antibody UCHL1 and OPD4 (both from Dako).

Langerhans cell enrichment from whole skin A fresh skin specimen ($\geq 4 \times 6$ cm) was placed into saline or 1×phosphate-buffered saline pH 7.4 at 4°C (on ice) prior to being cut into 0.5×2 cm strips. Strips were incubated dermal side down in 1% Dispase (Boehringer Mannheim) in Tyrode's salts solution (Sigma) at 4°C overnight. Following incubation at 37°C for half an hour, the epidermis was peeled away and rinsed in phosphate-buffered saline. Some of these epidermal strips were then processed directly for RNA extraction. They showed disruption through the basal layer with an intact suprabasilar region containing a normal complement of dendritic $CD1a^+$ Langerhans cells. No leukocytes or macrophages were detectable within the strips using anti-T cell (CD3), anti-B-cell (CD22), or anti-macrophage (CD14) monoclonal antibodies.

Some epidermal strips were floated dermal side down in 5 ml of 0.15% trypsin (bovine, Sigma) in phosphate-buffered saline and incubated at 37°C. After an initial 10 min period, the strips were agitated every 5 min for at least 30 min, not exceeding 1 h total incubation time. DNAse (Promega, Madison, WI) was added to a concentration of 3 units per ml, and the suspension was mixed 10 times with a pipet. The stratum corneum was aseptically removed, and an equal volume (5 ml) of complete RPMI was used to wash the cell suspension into a 15 ml tube. Cells were pelleted in an IEC HN-SII centrifuge for 8 min at 1100 rpm. The supernatant was discarded, and cells resuspended in 5 ml of complete RPMI 1640. The cell suspension was filtered through a 100 μm sieve, then passed through a 26 gauge needle, and filtered through a 38 μm sieve to obtain a single cell suspension. A sample was removed to check cell count and viability. Desired cell concentration was 5 million to 20 million cells per ml.

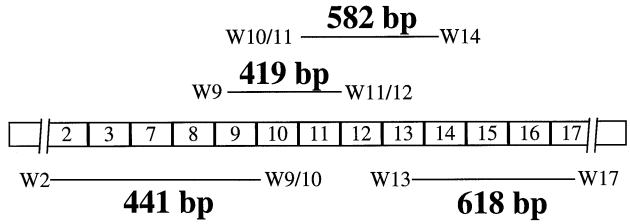

Figure 1. Schematic diagram of overlapping PCR primers used to determine the exon configuration of Langerhans cell CD45 cDNA.

Magnetic beads (Dynabeads M-450, Dynal, Lake Success, NY) were used in conjunction with unconjugated OKT6 (CD1a) monoclonal antibody (Ortho) to isolate the Langerhans cells. A modification of the direct method was used. The beads were washed with cold complete RPMI 1640 instead of phosphate-buffered saline/bovine serum albumin, both before and after addition, and incubation with, antibody (90 min at 4°C). The epidermal cell suspension was added to the washed, antibody-coated beads, and incubated for 90 min at 4°C with gentle agitation. This was followed by three washes with cold complete RPMI and final resuspension of beads/target cells (Langerhans cells) in cold complete RPMI. A sample was removed for cell counting, and appropriate numbers of Langerhans cells were transferred to Eppendorf tubes. The enriched Langerhans cells were 80%–90% pure with 10%–20% residual keratinocytes and no leukocytes or macrophages detectable by immunostaining of cytocentrifuge preparations with anti-T cell (CD3), anti-B cell (CD22), or anti-macrophage (CD14) monoclonal antibodies. The enriched Langerhans cells were pelleted, and supernatants decanted. The cell pellets were used for immediate RNA extraction or snap-frozen for future RNA extraction. Beads were not removed from the cells, but did not appear to interfere with RNA extraction.

CD45 oligonucleotide primers and internal probe The exon configuration of the entire extracellular domain of Langerhans cell CD45 was sequenced following reversetranscriptase/polymerase chain reaction (RT/PCR) amplification of the overlapping regions illustrated in **Fig 1**. The oligonucleotide primer pairs used to amplify these segments are listed in **Table II**.

RT/PCR Total RNA from isolated Langerhans cells and epidermal strips was recovered utilizing the acid guanidinium thiocyanate extraction method of Chomoczynski and Sacchi (1987) as described previously by us (Longley *et al*, 1991). Fresh or snap-frozen cells (10^4–10^6) were homogenized. DNA and proteins were removed with a salt/phenol/chloroform extraction, leaving the RNA in the aqueous phase. The RNA was precipitated with two volumes of 100% isopropyl alcohol at –20°C for a minimum of 1 h. The precipitate was pelleted by centrifugation and washed twice with 80% ethyl alcohol to remove any contaminating guanidinium and other salts. The pellet was vacuum dried and resuspended in sterile deionized water. The limited amount of RNA recovered was used directly for reverse transcription and amplification.

First-strand cDNA synthesis was carried out using Superscript RNAse H^- Reverse Transcriptase (Gibco BRL, Gaithersburg, MD) or Amv reverse transcriptase (Promega) and an appropriate downstream primer. Second-strand cDNA synthesis and subsequent amplification were accomplished using Ampli-Taq DNA polymerase (Perkin Elmer-Cetus, Branchburg, NJ) and appropriate primer pairs. Typically, amplification was performed for 30 cycles in a thermal cycler (denaturation × 1 min at 94°C, annealing × 1 min at 50–65°C, and extension at 60–72°C) at the highest possible temperatures within these ranges in order to minimize amplification of nontarget sequences. The RT/PCR product was screened on a 1% agarose gel and compared with molecular weight

Table II. CD45 oligonucleotide primer pairs and internal probe[a]

W2:	(–14)TTCCAGATATGACCATGTATTTGTGGC(13) uptream
W9/10:	(883)ACTTTTCAACCCCTGGTGGCACATCTAA(910) downstream
W9:	(773)GTGGAAACAATACTTGCACAAACAATGAGG(802) upstream
W11/12:	(1162)AAATAATCTGAGGCTCTCCTGGACTCCC(1189) downstream
W10/11:	(1007)TTACCTACAGATTTCAGTGTGG(1028) upstream
W14:	(1587)CTTATGCGACTCATTTCTAACC(1599) downstream
W13:	(1387)GCAAAAGTGCAACGTAATGGAA(1408) upstream
W17:	(1980)CCTTTATAGGAAACTTGCTGAACACC(2005) downstream
W7:	(612)GAGCCCTTCTGGAAGCGCTGTCATTTCAACCACAA(646) internal probe

[a]Sequence numbering according to Streuli *et al* (1987).

markers for determination of size and approximate quantity of amplified cDNA. The identity of the polymerase chain reaction (PCR) products was confirmed by Southern blot analysis (Sambrook *et al*, 1989) using a nested oligonucleotide DNA probe complementary to a portion of CD45 exon 7 (**Table II**).

DNA sequencing The PCR products obtained using primer pairs W2, W9/10 and W13, W17 were ligated into the pCRII vector (Invitrogen, San Diego, CA) based on overhanging A-T complementarity. The *Escherichia coli* strain INVαF (Invitrogen) was transformed with the vector and spread on LB/ampicillin plates containing X-GAl. Positive colonies were grown overnight in Luria broth containg ampicillin. Plasmid DNA was extracted and purified using Magic Maxipreps (Promega). Insertion of the target DNA was confirmed by *Eco* RI digestion. Target DNA was sequenced using standard autoradiographic methods (Sambrook, 1989) and using the AutoRead Sequencing Kit (Pharmacia LKB) that utilizes the Sanger dideoxy sequencing method (Sambrook, 1989) with fluoroscein labeled primers. Four-color sequence data were generated by the Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia LKB), and analyzed concurrently by computer.

The exon 2–9/10 and 13–17 regions amplified well using enriched Langerhans cells preparations; however, the region spanning exons 10–13 was more difficult to amplify despite the use of several alternative primers flanking this region (sequences not shown). We suspected that secondary structure might be interfering with efficient RT/PCR. Therefore, we modified our methods for this region. RNA was extracted from epidermal strips and amplified using W9, W11/12 and W10/11, W14 primer pairs. Products from multiple RT/PCR reactions were pooled, concentrated, digested with NlaIII to generate compatible ends with SphI-cut pUC 18 and pUC 19 vectors, and electrophoresed on Nusieve GTG agarose gels. Fragments of appropriate size were isolated, ligated into the vectors, cloned, and sequenced as described above.

RESULTS

The major Langerhans cell CD45 transcript lacks variable exons RNA extracted from epidermal strips and freshly enriched normal epidermal Langerhans cells was reverse transcribed into cDNA. We obtained better yields using a downstream primer rather than random hexamers. Because all known variability in CD45 primary structure originates from the presence or absence of the so-called variable exons (4, 5, and 6) (**Table I**), we focused our attention on the 5′ translated region of CD45 (exons 3–10). The 5′ portion of CD45 cDNA was PCR amplified using primers complimentary to exon 2 and exons 9–10 that flank the CD45 variable exon region (exons 4, 5, and 6) (**Fig 2**). A dominant 441 bp PCR product was amplified from both epidermal strips and enriched Langerhans cells along with small amounts of a larger 582 bp band. In contrast, similar amplification of peripheral blood mononuclear leukocytes produced the 441 bp band as well as larger amounts of multiple heavier bands. This was not unexpected for blood leukocytes that are known to produce multiple CD45 isoforms (Wood, 1991). It indicates that the dominance of the 441 bp product in Langerhans cells was not simply due to its shorter length. The similar findings from both epidermal strips and enriched Langerhans cells indicate that the Langerhans cells isolation process we employed did not significantly alter their expression of CD45 transcripts. The CD45 nature of these PCR products was confirmed by Southern blot analysis using a nested, exon 7-specific oligonucleotide probe.

The Langerhans cell PCR products were ligated into the pCRII vector (Invitrogen) based on overhanging A-T hybridization and cloned in *E. coli*. All 12 colonies tested contained the 441 bp insert except two that contained the 582 bp insert. These were sequenced by the dideoxy method using conventional sequencing gels and autoradiography (see **Fig 3**) as well as a computerized, automated nucleotide sequencer (Pharmacia) that utilizes a nonradioactive fluorochrome labeling system. Results were similar and revealed the following exon figuration for the 5′ translated region of the 441 bp product: exon 3/7/8/9/10. This confirmed our previous preliminary findings.[1]

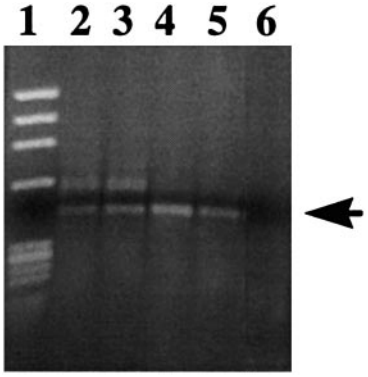

Figure 2. The dominant Langerhans cell CD45 RT/PCR product is 441 bp using primers W2 and W9/10. Mingel of RT/PCR products amplified using primers complementary to exon 2 and exon 9/10 that flank the CD45 cDNA variable region (exons 4–6). Langerhans cells (*lanes 4* and *5*) exhibit a dominant 441 bp band (*arrow*) whereas peripheral blood mononuclear leukocytes (*lanes 2* and *3*) exhibit multiple bands. Each of these lanes represents 100,000 cells except *lane 4* (240,000 cells). *Lane 1* is a ϕX174/*Hae* III digest. *Lane 6* is the carryover negative control.

All known isoform diversity within the human CD45 protein family arises from alternate splicing involving exons 4, 5, and 6 (Wood, 1991). Therefore, the remaining unsequenced extracellular domain of Langerhans cell CD45 was highly likely to have the same exon configuration as all other known isoforms. Nevertheless, in order to confirm this structure for Langerhans cell CD45, we sequenced the remainder of the extracellular domain extending well into exon 17, which encodes the first intracytoplasmic region of CD45 adjacent to the exon 16 transmembrane domain. Results showed an exon configuration identical to all other known isoforms of CD45: 10/11/12/13/14/15/16/17.

Normal epidermal Langerhans cells lack CD45R epitopes In aggregate, these sequencing studies showed that the major form of CD45 expressed by normal epidermal Langerhans cells has the same exon configuration spanning its entire extracellular domain (3/7/8/9/10/11/12/13/14/15/16/17) as the 180 kd CD45 isoform expressed by memory T cells and, more weakly, by monocytes. These cells, however, are $CD45RO^+$ whereas Langerhans cells in normal epidermis are generally $CD45RO^-$ (Wood, 1991). The CD45RO epitope is known to be an oligosaccharide with a terminal sialic acid moiety (Pulido, 1989; Poppema, 1991). Therefore, we determined the expression of a related epitope, OPD4, by Langerhans cells. This epitope also involves a terminal sialic acid moiety expressed by the 180 kd

[1]Wood GS, Crooks CF, Szwjebka P, Schwandt A: Human Langerhans cell CD45. *J Invest Dermatol* 100:560, 1993 (abstr.)

CD45 isoform of memory T cells, but not by monocytes (Pulido, 1989; Yoshido *et al*, 1989; Poppema, 1991). Fourteen tissue specimens were analyzed, including four normal skin (two breast, two nose), one reactive tonsil, two chronic spongiotic dermatitis, five mycosis fungoides (four patch/plaque, one tumor), one cutaneous lymphoid hyperplasia, and one cutaneous sinus histiocytosis (Rosai-Dorfman disease). In all samples, $CD1a^+$ Langerhans cells were $OPD4^-$. This included Langerhans cells in epidermis, cutaneous adnexal epithelium, and tonsil epithelium. In contrast, $CD3^+$ T cells in these specimens were $OPD4^+$ as expected. Representative findings are shown in **Fig 4**.

The minor Langerhans cell CD45 transcript contains variable exon 5 The larger 582 bp cloned PCR product was subjected to similar sequence analysis and exhibited the following exon configuration: 3/5/7/8/9/10. This was reminiscent of the 190 kd CD45 isoform expressed by certain leukocytes and associated with a $CD45RB^+$ phenotype in these cells.

DISCUSSION

In this study, we analyzed the structure of the 5′ translated regions of normal epidermal Langerhans cell CD45 transcripts. We identified a major 5′ transcript sequence with an exon 3/7/8/9/10 configuration and a minor 5′ transcript sequence with an 3/5/7/8/9/10 configuration. These results are consistent with our prior finding that Langerhans cells are $CD45RA^-$ (Wood, 1991) because expression of the CD45RA epitope is dependent upon synthesis of CD45 transcripts containing exon 4 (Ralph, 1987; Streuli *et al*, 1987, 1988, 1989; Hall, 1988). No RT/PCR products shorter than 441 bp were identified. This agrees with similar studies of thymocytes and T cell neoplasms, and indicates that variable splicing involving exons 7 and 8, which has been reported in mice, does not occur in these human cells (Ratech, 1997).

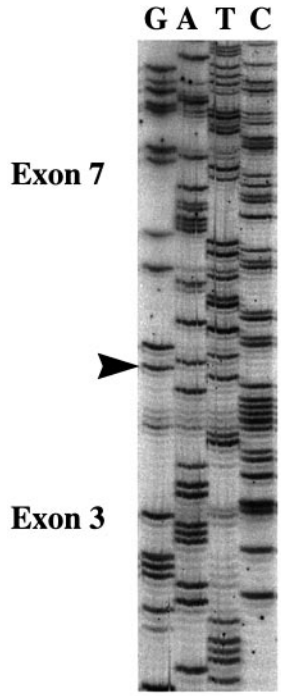

Figure 3. Sequencing shows that Langerhans cell CD45 contains the exon 3/7 junction. Nucleotide sequence of the Langerhans cells 441 bp RT/PCR product. Conventional sequencing gel autoradiogram illustrates the exon 3–exon 7 junction (*arrow*) with deletion of all intervening CD45 variable exons (exons 4–6).

The same exon configurations that we identified in Langerhans cell CD45 transcripts are associated with the 180 kd and 190 kd CD45 isoforms, respectively, that are expressed by various other leukocyte subsets (Ralph, 1987; Hall, 1988; Streuli *et al*, 1987, 1988, 1989). Among these other leukocytes, expression of the 180 kd isoform is associated with expression of the CD45RO epitope and expression of the 190 kd isoform is associated with expression of the CD45RB epitope; however, prior studies showed that Langerhans cells in normal epidermis are typically $CD45RB^-RO^-$ (Wood, 1991). The CD45RO epitope is expressed by memory T cells and monocytes and is known to be an oligosaccharide involving a terminal sialic acid moiety (Pulido, 1989; Poppema, 1991). Therefore, we studied Langerhans cells for expression of a related epitope, OPD4. The OPD4 epitope also involves a terminal sialic acid moiety and is expressed by the 180 kd CD45 isoform of memory T cells but is not expressed by monocytes (Pulido, 1989; Yoshido, 1989; Poppema, 1991; Macardle *et al*, 1995). Langerhans cells were $OPD4^-$; however, most intraepidermal and intradermal T cells were strongly $OPD4^+$.

Therefore, our data suggest that normal memory T cells, monocytes, and Langerhans cells all express a shared CD45 transcript lacking exons 4, 5, and 6; however, this transcript appears to undergo lineage-specific post-translational glycosylation to create three distinct CD45 glycoproteins: $CD45RO^+$ $OPD4^+$, $CD45RO^+$ $OPD4^-$, and $CD45RO^-$ $OPD4^-$ that are expressed by memory T cells, monocytes, and Langerhans cells, respectively. Because these epitopes are located extracellularly, we postulate that they allow differential responses to extracellular stimuli by creating differential ligand specificity.

These findings are important because they indicate a previously unrecognized mechanism for CD45 molecular diversity. A similar system of cell type-specific and developmental stage-specific variation in glycosylation has been described for CD43, another membrane-associated, glycoprotein widely expressed by leukocytes (Peacocke, 1992). The potential for structural diversification inherent in the differential glycosylation of proteins can be appreciated by recalling that the linking of two amino acids can create only two unique structures (A-B or B-A), whereas the linking of two hexose sugars can create 16 unique structures. Because memory T cells retain strong expression of CD45RO and OPD4 epitopes when they infiltrate the epidermis, the intraepidermal microenvironment, per se, cannot be presumed to exert a downregulatory effect on, or masking, CD45RO and OPD4 expression by leukocytes. Their absence from Langerhans cells appears to be lineage specific as opposed to microenvironment specific. CD43, CD44, and CD45 molecular weight in EL-4 thymoma

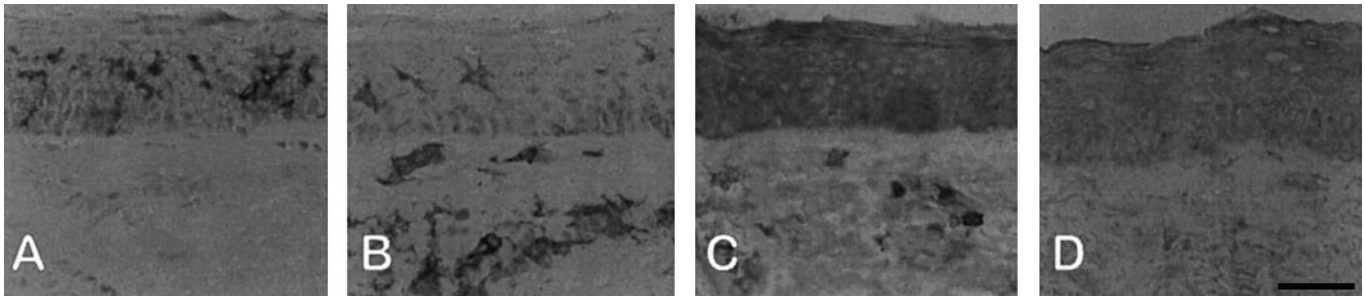

Figure 4. Langerhans cells *in situ* lack expression of both UCHL1 and OPD4 epitopes. Immunoperoxidase stained frozen sections of skin show that epidermal $CD1a^+$ Langerhans cells (*A*) express CD45 common epitope 2D1 (*B*) but lack expression of the CD45RO-related epitopes UCHL1 (*C*) and OPD4 (*D*). Dermal histiocytes and lymphocytes reactive with 2D1 (*B*) and UCHL1 (*C*) are also present, as well as a single dermal lymphocyte stained by OPD4 (*D*). Methylene blue counterstain; *scale bar*: 30 μm.

cells can be increased by transfection with core 2 β 1→6 N-acetylglucosaminyltransferase, which increases the branching of O-linked glycans (Baran *et al*, 1997). It is possible that lineage-specific, activation-specific, or maturation stage-specific differences in the activity of such enzymes involved in the O-glycan biosynthetic pathway might help explain the unique structure of Langerhans cell CD45 in normal human epidermis.

Support for this concept comes from a prior study (Bieber *et al*, 1995) that showed that freshly isolated normal epidermal Langerhans cells could alter their CD45R expression *in vitro* over time or in response to cytokines. The authors postulated that their isolation and culture of Langerhans cells induced activation events promoting Langerhans cell differentiation or maturation accompanied by a shift in CD45R phenotype. These findings are consistent with our earlier observation that peripheral blood dendritic cells differ from epidermal Langerhans cells in their CD45 phenotype. Like epidermal Langerhans cells, they express high levels of CD45 common epitopes; unlike epidermal Langerhans cells, they express low levels of CD45RO and moderate levels of CD45RB (Wood, 1991).

In concurrence with our current and past findings (Wood, 1991) concerning the CD45 phenotype of normal Langerhans cells in intact epidermis, Bieber *et al* (1995) also found that freshly isolated Langerhans cells expressed CD45 common epitopes but lacked CD45RA and CD45RB. It is possible that alterations induced by their Langerhans cells enrichment procedure (Bieber *et al*, 1989) might explain why they found that a majority, but not all, of their freshly isolated Langerhans cells expressed some CD45RO, whereas we found that Langerhans cells in intact normal epidermis typically lack CD45RO. Differential sensitivity of the detection methods used (three-stage immunoperoxidase staining *versus* two-stage fluorescence-activated cell sorter analysis) is unlikely to account for this discrepancy because we previously observed a good correlation between these two methods when applied to the immunophenotyping of dendritic cells (Wood, 1991, 1992; Freudenthal, 1993). In addition, Langerhans cells in intact epidermis also lacked CD45RO using sensitive two-color *in situ* immunofluorescence techniques (Wood, 1991). In aggregate, our data indicate that Langerhans cells in intact epidermis express little or no CD45RO. Nevertheless, even if they expressed very low level CD45RO *in situ*, it would have to be far less than their expression of CD45 common antigens. At the very least, this implies novel hypoglycosylation of Langerhans cell CD45 framework proteins relative to other cells, such as T cells, that strongly express both CD45 common and CD45RO epitopes whether assessed by *in situ* immunoperoxidase, *in situ* immunofluorescence, or fluorescence-activated cell sorter analysis. Unfortunately, Bieber *et al* presented no data concerning the absolute or relative intensity of the CD45RO expression that they detected in a subset of their isolated Langerhans cells.

The minor CD45 transcript that we detected in Langerhans cells had an exon 3/5/7/8/9/10 configuration identical to the 190 kd CD45 isoform expressed by other leukocytes and usually associated with expression of the CD45RB epitope (Ralph *et al*, 1987; Streuli *et al*, 1987, 1988, 1989; Hall, 1988). Because intact epidermal Langerhans cells and freshly isolated Langerhans cells are typically $CD45RB^-$ (Wood, 1991; Bieber, 1995), and the CD45RB antigen is known to be another O-linked oligosaccharide with a terminal sialic acid moiety (Pulido, 1989), this may represent another example of lineage-specific differences in glycosylation. Alternatively, because this product was relatively rare, it might represent very low-level expression of the conventional $CD45RB^+$ isoform containing exon 5 but lacking exons 4 and 6. In this case, its antigen density would be too low to generally detect using conventional immunophenotyping methods.

In addition to their demonstrated value in analyzing the structure of CD45 isoforms, the techniques employed in this study should be equally useful for investigating the structure of other molecules expressed by Langerhans cells or other rare cell types. Once the cells of interest are enriched and depleted of any relevant contaminating cell types, sequence analysis of RT/PCR products can be used to determine the structure of gene transcripts and thereby deduce the primary structure of corresponding protein products. Provided that an appropriate panel of antibodies is available, prosthetic groups attached to these proteins can be further characterized by epitope mapping studies performed on tissue sections or cell suspensions. Together, these methods can define a wide variety of protein structural features. They are especially useful for studying proteins expressed by rare cells that are difficult to harvest in numbers adequate for analysis of mRNA by northern blotting or analysis of proteins by western blotting or immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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